

Effect of Purple Sweet Potato (*Ipomoea Batatas* L.) on Reducing Renal Tissue Damage of House Mice (*Mus Musculus* L.) After Excessive Physical Exercise

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ABSTRACT

Background: Heavy physical exercise can reduce blood flow and metabolism in kidney that eventually release free radicals. The free radicals can form oxydative stress and damage renal tissue. Exogenous antioxydant administration is usually recommended to minimize the renal tissue damage. This study aimed to examined the effect of purple sweet potato (*Ipomoiea batatas* L.) extract on renal tissue damage in mice (*mus musculus* L.) after heavy physical exercise.

Subjects and Method: This was a randomized controlled trial with post test only control design. The study subjects included twenty four male white mice with DD Webster strain. These mice were divided into six groups. After undergoing excessive swimming exercises that lasted fourteen days, purple sweet potato extract was given to the experimental group. There were three experimental groups receiving three different doses of purple sweet potato. The mice kidney was taken as sample for microscopic examination to determine the extent of tissue damage. Difference in renal tissue damage was tested by Kruskal-Wallis.

Results: Microscopic examination showed statistically significant difference in tissue damage both in right ($p=0.001$) and left ($p=0.036$) kidneys, between study groups. The experimental groups showed less damaged than control group.

Conclusion: Purple sweet potato (*Ipomoiea batatas* L) can lessen renal damage in male white mice (*Mus musculus* L) undergoing excessive physical exercise.

Keywords: purple sweet potato (*Ipomoiea batatas* L), renal tissue damage mice

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BACKGROUND

Physical exercise can increase oxygen consumption since there is metabolism escalation within body such as in muscle, heart, and brain. In the other side, bloodstream and metabolism decline significantly on heart and kidney during exercise (Radak et al., 2013).

The bloodstream reduction in kidney generates the emergence of ischemia-reperfusion which will activate xanthine oxidase system. The process of ischemia-reperfusion

and leucocytes activation through oxidase NADPH system may cause oxidative stress to kidney during and after exercise. Both mechanisms are extremely responsible for the emergence of oxidative stress inside the organ and extramuscular tissue after physical exercise (Kocer et al., 2008).

Oxidative stress is imbalance between reactive oxygen compounds and antioxidants (Urso dan Clarkson, 2003). Reactive oxygen compounds are produced from oxygen molecule as the result of normal cell

metabolism. The three major reactive oxygen compounds that possess physiology effect are superoxide anion (O_2^-), hydroxyl radicals ($OH\cdot$), and hydrogen peroxide (H_2O_2) (Birben et al., 2012). Commonly 2-5% of oxygen used by mitochondria will form free radicals (Urso dan Clarkson, 2003).

When metabolism demands escalate as it is in physical exercise, cells may endure relative hypoxia though bloodstream is normal in several organs including kidney. Hypoxia on tubule cell of kidney can generate the emergence of apoptosis on cell (Nangaku, 2006). Okolow et al., (2006) found that intensive physical exercise could induce apoptosis in distal tubule cell of mice that undergo physical exercise which was treadmill until they were exhausted for the whole ninety minutes. After the physical exercise finished, the blood would rapidly go back to kidney and along with it big amount of oxidant was released (Daniel et al., 2010). Oxidative stress and relative hypoxia may cause damage to kidney cell.

More than 40% of people who conduct physical exercise consume antioxidant supplement to maintain health (Bucioli et al., 2011). Nangaku (2006) conveyed that antioxidant is one of the medicines that targets hypoxia on kidney by repair cellular respiration process.

One of the plants in Indonesia that contains quite amount of antioxidant is purple sweet potato (*Ipomoea batatas* L.). The purple color on the tuber is the result of a compound known as anthocyanin and functions as antioxidant. Tuber of purple sweet potato contains pretty much of anthocyanin that is around 519 mg/ 100 g wet weight (Richana, 2013). Anthocyanin is able to act as antioxidant directly by donating electron or transfer hydrogen atom from hydroxyl cluster to free radicals (Prior, 2003) and may bind with reactive oxygen species (ROS) such as superoxide

(O_2^-), singlet oxygen (1O_2), peroxide ($ROO\cdot$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH\cdot$) (Pojer et al., 2013).

Based on the elaboration above it can be concluded that physical exercise may lead to the occurrence of oxidative stress and relative hypoxia to kidney which eventually may damage kidney cells. Since purple sweet potato is one of the sources of good antioxidants, therefore it need to conduct a study on the effect of purple sweet potato extract toward histopathology of mice's kidney after maximal physical exercise.

SUBJECTS AND METHOD

The sample of the study was male white mice (*Mus musculus* L.), Strain DD Webster, aged 8-9 weeks weighed 25-35 g obtained from Faculty of Mathematics and Natural Science, North Sumatra University. The mice were acclimatized for one week in laboratory animals' cage and being fed also given drink as much they need. The room's light was controlled exactly at 12 hours of light (06.00-18.00) 12 hours of dark (18.00-06.00), whereas temperature and humidity were allowed to be in its natural range. "Ethical Clearance" was obtained from ethic committee of Faculty of Mathematics and Natural Science, North Sumatra University for the use and treatment of the laboratory animals. The amount of the sample was 30 mice.

Research Design

The research was purely experimental study with post test only control group design as the research design. The samples were divided into 6 groups, each consisted of mice. The first group was the control group and was not given any treatment only food and drink in ad libitum. The second group obtained physical exercise with maximally 60 minutes of swimming and orally 0.5 ml of aquabidest/ mouse/ day. The third group

orally obtained 0.5 ml of purple sweet potato tuber extract/ mouse/ day. The fourth group obtained physical exercise 60 minutes of swimming at the maximum and orally 0.5ml of purple sweet potato tuber extract/ mouse/ day.

The fifth group obtained physical exercise 60 minutes of swimming at the maximum and orally 1ml of purple sweet potato tuber extract/mouse/day. The sixth group obtained physical exercise 60 minutes of swimming at the maximum and orally 1.5 ml of purple sweet potato tuber extract/ mouse/day. The drenching of aquabidest and purple sweet potato tuber extract was administered 3 hours after swimming. All treatments was conducted for 14 days. Up to the 14th day one mouse of each group died (6 mice died as a whole) therefore only 24 mice which were decapitated and the kidneys were taken.

Maximum Physical exercise

Exercise in which mice conducted swimming activity with all their might until they almost drowned or they seemed to be exhausted as their entire body almost drowned except for nose, and their limbs were weakened. Swimming was conducted for 60 minutes wherein the swimming time was taken from the result of preliminary test conducted prior to the research.

Extract Making

Tubers of purple sweet potato were rinsed with clean water, peeled out and chopped crosswise with 2-2.5 cm of thickness. The chopped tuber tubers were steamed for \pm 15 minutes until tender, and kept from being cracked or split. Afterward the tubers were cooled and put in a certain place to be fermented by adding wine yeast (tape starter) that can be bought in traditional market. Fermentation was conducted for 36 hours and the result was mixed with clean drinking water by comparison 1kg of tape (fermented tubers) added with 1 liter of water

then processed with blender afterward screened with 3 layers of gauze. The result was given to the mice. The level of anthocyanin was measured by using spectrophotometer on 520 nm and 720 nm. From the measurement it was obtained the level of anthocyanin in fresh purple sweet potato tuber was 9,984 mg/100 g and in fermented tuber was 89,666 mg/100 g.

Histopathology of Kidney

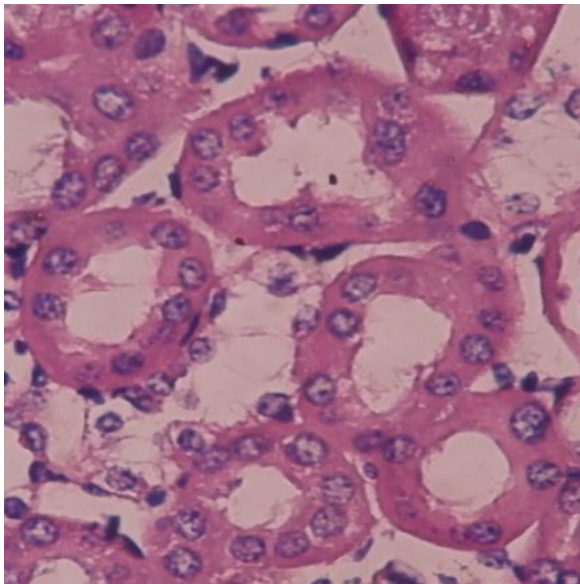
Kidney was fixated by using 10% buffered formalin solution and paraffinized. Paraffin block which was cut then colored using Haematoxylin-Eosin (H-E), observation was conducted under light microscope with magnification 40x and 400x. The observation was conducted by dividing preparat into 5 parts, every part was observed on how extensive damage of kidney tubule in the form of hydrofic degeneration and necrosis. Hydrofic degeneration was marked by cell inflamemation because of the accumulation of liquid inside the cytoplasm. Necrosis was marked by the occurrence of nucleus degenartion in the from of Karyopiknosis (small and solid nucleus), Karyolysis (pale and dissolved nucleus) dan Karyoreksis (nucleus was torn into some lumps).

The percentage of damage extensiveness on kidney tubule from the 5 parts then it was sum up and divided by five. Afterwards the level of damage on kidney tubule was measured with criteria of Santoso dan Nurliani in Manurung (2011) which was modified as follow: 0= Normal: if there is no hydrofic degeneration and necrosis found, 1= Mild: if there is hydrofic degeneration found, 2= Moderate: if the extensiveness of kidney tubule necrosis found <25%, 3= Severe: if the extensiveness of kidney tubule necrosis is 26%-50%, 4= Very severe: if the extensiveness of kidney tubule necrosis is >50%.

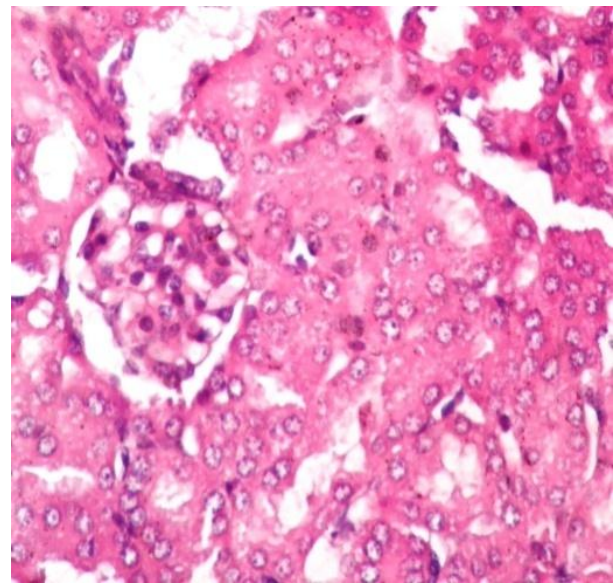
The distribution of data obtained from the previous study was determined by Normality test and also Homogeneity test. If the data was normally and homogeneously distributed then it was followed by ANOVA. If the data was not normally distributed then Kruskal-Wallis would be conducted. All data analysis was conducted by using SPSS 19 software. In this study the determination of statistic test was taken by significant level 5% ($p=0.05$) that was considered meaningful or significant.

RESULTS

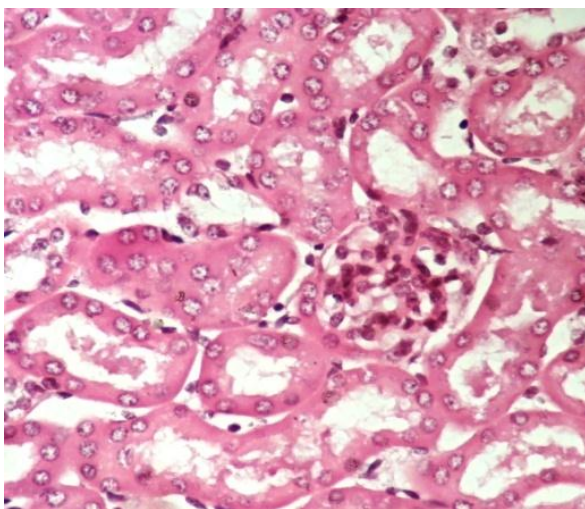
The result of examination by using microscope obtained that kidney tubules in control group did not endure damage or they are still normal, whereas in group which received treatments the kidney tubules were damage that was necrosis with the karyolysis that was nucleus became pale and dissolved. Necrosis in kidney tubules possessed various extensivenesses. Damages in kidney tubules can be observed microscopically in Picture 1.



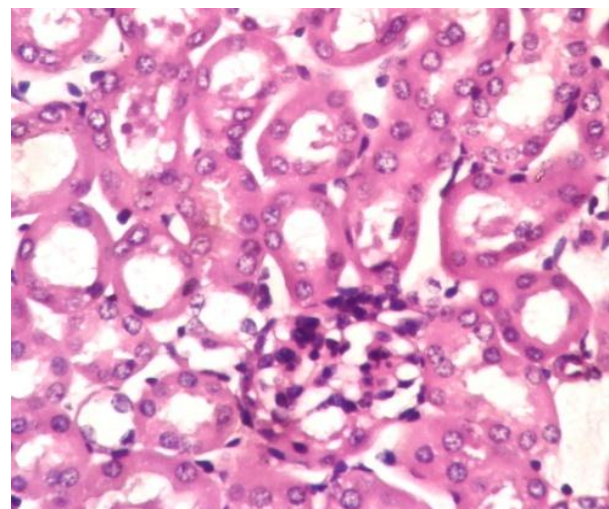
A



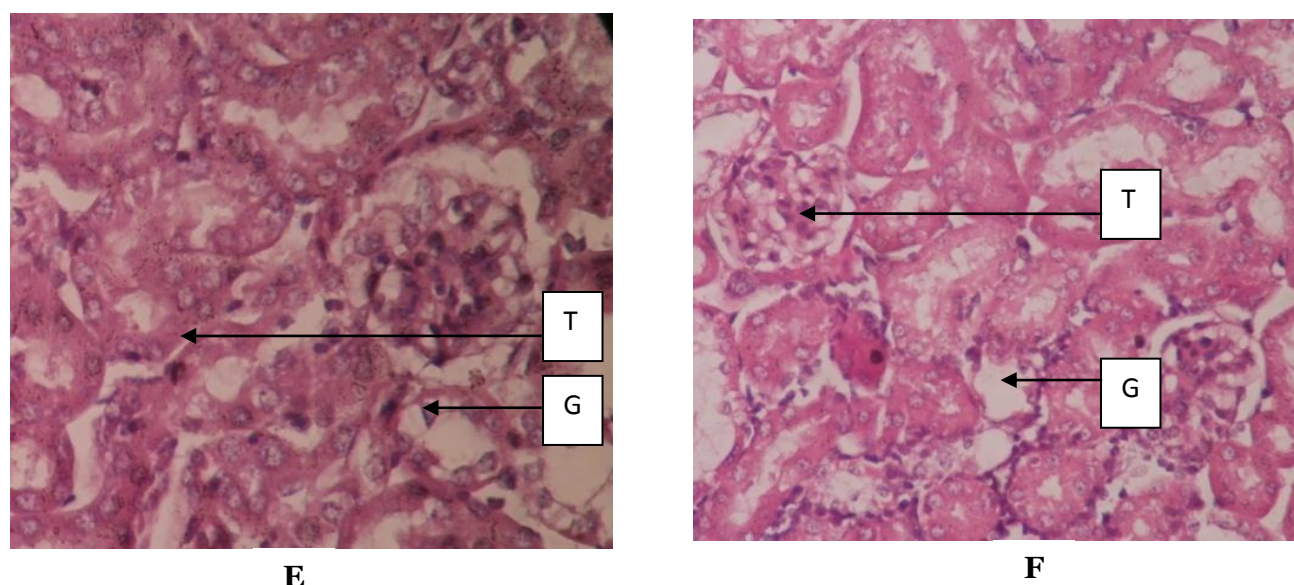
B



C



D



Picture 1. Kidney microscopic image. Kidney within normal limit is in group P1(1A). Kidney tubule damage (T) in the form of necrosis (karyolysis) is in P2 (1B), P3 (1C), P4 (1D), P5 (1E) dan P6 (1F). Glomerulus (G) is within normal limit (HE 400X).

The result of the research obtained that right kidney tubules in group P1 did not endure damages or they were normal. Group P2 endured damages that were included in very severe category. Group P3 and P4 endured damages categorized as

moderate and severe. Group P5 endured damages categorized as severe and group P6 endured damages categorized as severe and very severe, as it is presented in Table 1.

Table 1. Degree of right kidney tubules damages in microscopic

Group	Degree of right kidney tubules damages								Total		p
	Normal		Moderate		Severe		Very severe				
	n	%	n	%	n	%	n	%	n	%	
P1	4	100	0	0	0	0	0	0	4	100	0.001
P2	0	0	0	0	0	0	4	100	4	100	
P3	0	0	3	75	1	25	0	0	4	100	
P4	0	0	2	50	2	50	0	0	4	100	
P5	0	0	0	0	4	100	0	0	4	100	
P6	0	0	0	0	2	50	2	50	4	100	

Kruskal-Wallis test

Information: (P1) Control; (P2) Maximal physical exercise; (P3) Extract 0.5 ml; (P4) Maximal physical exercise + extract 0.5 ml; (P5) Maximal physical exercise + extract 1 ml; (P6) maximal physical exercise + extract 1.5 ml.

The data were not distributed normally and data variants were not similar therefore Kruskal-Wallis test was conducted. In Kruskal-Wallis test presented in Table 1 the

value of $p=0.001$ is obtained. It means there is meaningful difference on the histopathology of mice's right kidney tubules in

control group compared to group that received no treatment.

The result of the study showed that left kidney tubules in group P1 did not endure damage or they were normal. Group P2 endured damages that were categorized

as severe and very severe. Group P3, P4, P5 endured damages that were categorized as moderate, severe, and very severe. Group P6 endured damages that were categorized as severe and very severe, as it is presented in Table 2.

Table 2. Degree of left kidney tubules damages in microscopic

Group	Degree of left kidney tubules damages								Total	p
	Normal		Moderate		Severe		Very Severe			
	n	%	n	%	n	%	n	%	n	%
P1	4	100	0	0	0	0	0	0	4	100
P2	0	0	0	0	3	75	1	25	4	100
P3	0	0	1	25	2	50	1	25	4	100
P4	0	0	1	25	2	50	1	25	4	100
P5	0	0	1	25	2	50	1	25	4	100
P6	0	0	0	0	2	50	2	50	4	100

Kruskal-Wallis test

Information: (P1) Control; (P2) Maximal physical exercise; (P3) Extract 0.5 ml; (P4) Maximal physical exercise + extract 0.5 ml; (P5) Maximal physical exercise + extract 1 ml; (P6) maximal physical exercise + extract 1.5 ml.

The data were not distributed normally and data variants were not similar therefore Kruskal-Wallis test was conducted. In Kruskal-Wallis test presented in table 1 the value of $p = 0.036$ is obtained. It means there is meaningful difference on the histopathology of mice's right kidney tubules in control group compared to group that received no treatment.

DISCUSSION

From the study result it was obtained that left and right kidney tubules in control group did not endure damages or they were normal. Damages on left and right kidney tubules occurred on all treated groups. The damages were marked with the emergence of necrosis with various of extensiveness.

Damages on cells may occur reversible and irreversible. Reversible damages are marked by the emergence of swelling dan transformation on cell fat. Swelling occurred because plasma membrane fails in pumping ion consequently ion and liquid

homeostasis is disrupted (Kumar et al., 2007). Cell fat transformation occurred because of the emergence of hypoxia which is marked by the emergence of fat vacuoles, small or big in cytoplasm (Kumar et al., 2007)

Irreversible damages are marked by necrosis. Necrosis is emerged for there is enzyme degradation in cells (dissolution) inti sel. Necrotic cells are not able to maintain membrane unity, therefore the content of the cell often comes out. By using electron microscope necrotic cells are marked with the emergence of: damage of plasma membrane and organelle membrane; the mitochondria dilatation with the emergence of big density that is in amorphous shape; the disruption of lysosomes and the transformation of cell nucleus that ends up with the damage (dissolution) of cell nucleus.

Tubules damage is lead by the occurrence of oxidative stress during maximal physical exercise. Oxidative stress as the result of the exercise may cause muscle damage

and interfere some tissues including heart, kidney, liver, brain, and erythrocytes. The source of oxidative stress that works in kidney may be based on two enzyme systems that are leukocytes activation (system of NADPH oxidase enzyme) and process of ischemic-reperfusion (system of xanthine oxidase enzymes) that are source of ROS produced by extramuscular tissues during exercise (Kocer et al., 2008). ROS is identified as the cause of cell injury possibility in many diseases.

In addition of two enzyme systems, tubules damage is also caused by the occurrence of relative hypoxia in kidney. When metabolism needs increasing such as during physical exercise, cells may endure relative hypoxia although bloodstream is normal in several organs including kidney. Hypoxia or the lack of oxygen, may disrupt aerobic oxidative respiration and is the important and common cause of cell injuries and deaths (Kumar et al., 2007).

In this study, the administration of purple sweet potato tuber that contained anthocyanin could not restore cells back to normal. The result of the study was different to Sreedevi dan Pavani (2012) who found that anthocyanin administration might restore necrosis in kidney tubules of male albino rats that were injected with cisplatin.

Based on the result of the study it was concluded that the extract of purple sweet potato tubers (*Ipomoea batatas* L.) could not restore the cells back to normal in kidneys of male mice (*Mus musculus* L.) that were given maximal physical exercise. A further study needs to be conducted to consider the level of alcohol formed during the extract making through fermentation.

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